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# **Recovery following a marathon: A comparison of cold water immersion, whole body cryotherapy and a placebo control**

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## ABSTRACT

**Purpose:** Cryotherapy is an increasingly popular recovery strategy used in an attempt to attenuate the negative impact of strenuous physical activity on subsequent exercise. Therefore, this study aimed to assess the effects of whole body cryotherapy (WBC) and cold water immersion (CWI) on markers of recovery following a marathon.

**Methods:** Thirty one endurance trained males completed a marathon. Participants were randomly assigned to a CWI, WBC or placebo group. Perceptions of muscle soreness and training stress, and markers of muscle function were recorded before the marathon, and at 24 and 48h post exercise. Blood samples were taken at baseline, post intervention, and 24 and 48h post intervention to assess inflammation and muscle damage.

**Results:** WBC had a harmful effect on muscle function compared to CWI post marathon. WBC positively influenced perceptions of training stress compared to CWI. With the exception of C-Reactive Protein (CRP) at 24h and 48h, neither cryotherapy intervention positively influenced blood borne markers of inflammation or structural damage compared to placebo.

**Conclusion:** The findings show WBC has a negative impact on muscle function, perceptions of soreness and a number of blood parameters compared to CWI, contradicting the suggestion that WBC may be a superior recovery strategy. Further, cryotherapy is no more effective than a placebo intervention at improving functional recovery or perceptions of training stress following a marathon. These findings lend further evidence to suggest that treatment belief and the placebo effect may be largely responsible for the beneficial effects of cryotherapy on recovery following a marathon.

**Keywords:** Muscle Damage; Placebo; Muscle Function; Inflammation; Endurance

## Abbreviations

CK	Creatine Kinase
CMJ	Counter Movement Jump
CRP	C-Reactive Protein
CWI	Cold Water Immersion
DALDA	Daily Analysis of the Lifestyle Demands of Athletes
EIMD	Exercise Induced Muscle Damage
IL-6	Interleukin-6
MVIC	Maximal Voluntary Isometric Contraction
RSI	Reactive Strength Index
TNF- $\alpha$	Tumour Necrosis Factor- $\alpha$
WBC	Whole Body Cryotherapy

## INTRODUCTION

Across both recreational and elite level sport, athletes regularly train or even compete multiple times a week. It is well documented that novel or exhaustive exercise, whether mechanical or metabolic in nature, can result in exercise induced muscle damage (EIMD) (Belcastro, Shewchuk, & Raj, 1998) and inflammation (Pyne, 1993). These physiological stresses can manifest as reduced performance potential, likely to result from increased muscle soreness (Cheung, Hume, & Maxwell, 2003) and decreased muscle function (Byrne & Eston, 2002), as well as increased stiffness and swelling that can last for a number of days following the initial insult (Armstrong, 1984).

Where performance is a crucial consideration, the optimisation of recovery in between exercise bouts to minimise any negative impact on subsequent performance is vital (Barnett, 2006). Cryotherapy, either in the form of cold water immersion (CWI) or whole body cryotherapy (WBC), is becoming an increasingly popular recovery strategy employed by athletes (Hohenauer, Taeymans, Baeyens, Clarys, & Clijsen, 2015). Changes in physiological mechanisms resulting from a decrease in muscle and/or skin temperature include; reduced inflammation, analgesia, reductions in cardiovascular strain, decreased blood flow, reduced tissue metabolism, increased removal of muscle metabolites as well as neuromuscular, cardiovascular and hormonal changes (Ihsan, Watson, & Abbiss, 2016; Leeder, Gissane, van Someren, Gregson, & Howatson, 2012). It is likely that any performance effects resulting from a cryotherapy intervention could be attributed to one, or a combination, of these physiological phenomena.

Despite the growing body of literature, there is still a lack of clarity regarding the efficacy of CWI and WBC as recovery strategies. This may be due in part to the fact that the type and magnitude of physiological stress experienced following a bout of exercise is heavily dependent on the specific nature and duration of the exercise. Evidence suggests that CWI can attenuate soreness (Bleakley et al., 2012; Hohenauer et al., 2015; Leeder et al., 2012) following a variety of exercise stressors but the effect on muscle function remains less clear (Bleakley et al., 2012; Hohenauer et al., 2015). However, there is conflicting evidence to demonstrate that CWI has no effect on soreness (Leeder et al., 2015) following repeated sprints. Similarly, WBC has been shown to attenuate soreness following metabolic and mechanical stress (Hausswirth et al., 2011), but recent evidence suggests that ambiguity remains in the literature (Costello et al., 2015). However, there is little evidence to support improvements in functional recovery (Bleakley, Bieuzen, Davison, & Costello, 2014). It is likely that the equivocal results are due to differences in temperature, timing of application, type of exercise stress and training status of participants (Minett & Costello, 2015).

The rise in popularity of WBC as an alternative to CWI may be explained in part by the capacity to attain far lower exposure temperatures, possibly offering enhanced benefits to recovery. It has been proposed that cryotherapy has the potential to limit inflammation by decreasing peripheral blood flow and therefore limiting migration of inflammatory cytokines to areas of structural damage (Mawhinney et al., 2017). However, although WBC produces greater temperature gradients for tissue cooling, the relatively poor thermal conductivity of air compared to water limits the potential for significant subcutaneous and core body cooling (Bleakley et al., 2014). This is supported by both Costello et al., (2014) and Mawhinney et al., (2017) who demonstrated that CWI exposure elicits greater reductions in skin and tissue temperature than WBC. Despite a growing body of literature, there are still relatively few studies that directly compare WBC and CWI (Abaïdia et al., 2016; Mawhinney et al., 2017). Further research is required to afford researchers better understanding of the circumstances under which either treatment is, or is not effective, and whether one intervention can offer any substantial benefit over the other.

To date only one study has directly compared the efficacy of CWI and WBC on functional recovery. Abaïdia et al., (2016) compared the effects of CWI (10 min at 10°C) and WBC (3 min at -110°) on recovery following eccentric single-leg hamstring exercise. Their results demonstrated that there was a very likely moderate effect in favour of CWI for recovery of single and double leg CMJ compared to WBC 72 hours post exercise. Further, CWI elicited a moderate reduction in perceived soreness and a moderate increase in perception of recovery at 24 and 48h post respectively. The authors concluded that CWI was more effective than WBC in enhancing recovery of CMJ 72 hours after exercise. However, the exercise stress utilised in this study lacks ecological validity, and there was no control group for comparison.

Given the trend for increasing use of WBC despite equivocal research relating to both CWI and WBC, it is pertinent to make direct comparisons between the two modalities to investigate whether one method can offer a considerable advantage over the other. There are currently only a handful of studies directly comparing WBC and CWI and methodological differences make it difficult to draw cross study comparisons; the need for specificity in post-exercise recovery strategies has previously been highlighted by Minett & Costello (2015) and Stephens et al., (2016).

Long duration endurance exercise such as marathon running results in alterations of a number of physiological and perceptual parameters including muscle soreness, muscle function, muscle damage (CK) and inflammation (CRP) (Hill, Howatson, van Someren, Walshe, & Pedlar, 2014; Shanely et al., 2013). Whilst a number of studies have utilised CWI as a means of rapid cooling in the treatment of exertional heatstroke following

marathon performance (Casa et al., 2007; Mcdermott et al., 2009), there appears to be little evidence evaluating the efficacy of CWI or WBC on recovery following a marathon.

Therefore the aim of this study was to investigate the effects of WBC and CWI on both physiological and perceptual parameters of recovery in trained runners following the completion of a marathon run.

## **MATERIALS AND METHODS**

### **Participants**

31 healthy male volunteers participated in this study (Table 1). Participants were trained endurance runners and had an expected completion time of 4.5 hours or less for a marathon. All participants were non-smokers with no history of recent illness or other disease. In the five days prior to the run and for the duration of the study, participants were asked to abstain from therapeutic treatments including massage and anti-inflammatory drugs (NSAID), as well as any nutritional supplements. Participants were instructed to refrain from strenuous exercise (other than the marathon itself) for at least 2 days before each testing session.

### **Study Design**

All procedures were granted ethical clearance by the Institutional committee according to the Helsinki declaration prior to testing. Participants received both verbal and written information about the purpose and potential risks of all study procedures. Participants gave their written informed consent and completed a comprehensive health questionnaire, before being randomly assigned into the placebo (n=10), CWI (n=11) or WBC (n=10) intervention group. On the first testing day and prior to the marathon run participants were familiarised with all testing procedures before baseline measures of all dependent variables (DVs) were recorded. Participants began their allocated treatment intervention within 15 minutes of cessation of exercise, and then provided a further blood sample for analysis. Repeat measurements of all DVs were recorded at 24h and 48h following completion of the run. Data collection took place over a number of months and environmental conditions were recorded for each marathon day. Participant characteristics, completion times and environmental conditions are included in table 1.

Table 1. Participant characteristics, completion times and environmental conditions.

	Age (yr)	Height (cm)	Body Mass (kg)	Marathon PB (hh:mm:ss)	Completion Time (hh:mm:ss)	Max Temp (°C)
PL	40.6 ± 7.2	174.7 ± 8.6	75.9 ± 10.2	03:20:27 ± 00:25:19	03:40:18 ± 00:33:08	18.4 ± 3.7
CWI	41.3 ± 7.6	178.3 ± 7.6	79.2 ± 10.2	03:33:33 ± 00:27:10	03:43:05 ± 00:13:42	19.1 ± 4.2
WBC	37.7 ± 8.9	178.0 ± 5.2	77.8 ± 6.9	03:36:51 ± 00:18:48	03:59:06 ± 00:17:13	17.8 ± 1.8

Values are presented as mean ± SD

Max Temp refers to average maximum temperature recorded on the baseline testing days for each intervention

### Exercise Protocol

Participants completed the marathon in North London and were asked to pace the run as if it were a competitive race. The route was predominantly grass and unpaved footpaths, with some short concrete sections. Participants completed 9 laps of a 4.7 km loop which was marshalled at the start/finish point. Participants were allowed to consume fluids, electrolytes and/or food ad libitum during the run but were asked to avoid consuming any supplements containing BCAAs, protein, antioxidants or caffeine. By using an outdoor route and self-selected pace, it was hoped that the run would more closely mimic a real world scenario than a treadmill based protocol.

### Dependent Variables

#### Peak Torque and Isometric Contractions

Peak knee extensor torque and maximal voluntary isometric contraction (MVIC) were measured on the self-reported dominant limb using an isokinetic dynamometer (Biodex 3, Biodex Medical Systems, Shirley, NY, USA). Following a standardised warm-up participants performed 3 maximal efforts at 60 deg·s<sup>-1</sup>. Participants were encouraged to work as fast and as hard as possible against the resistance of the dynamometer arm throughout the full range of motion. MVIC was measured at a knee angle of 90° in accordance with previous studies (de Ruiters, van der Linden, van der Zijden, Hollander, & de Haan, 2003). Participants completed 3 maximal 5 second efforts. Peak values were used for analysis.

#### Drop Jump (DJ)

Participants dropped from a platform at a height of 30 cm onto a portable jump mat (Kinematic Measurement System, KMS, Fitness Technology, Australia) and then jumped vertically for maximum height as quickly as possible. Emphasis was placed on minimum ground contact time, whilst maintaining maximum jump height. Participants kept their hands on their hips for the duration of the movement, and performed 3 maximal jumps at each testing point. Reactive strength index (RSI) for each effort was calculated by dividing vertical



displacement (jump height) in metres, by ground contact time in seconds (Flanagan & Comyns, 2008) and peak RSI values were used for analysis.

### **Perceived Soreness**

Participants indicated their perceived levels of muscle soreness of the lower limbs during a body weight squat (approx. knee angle of 90°) using a 0 (no soreness on movement) to 10 (muscles too sore to move) likert scale. This method has been used successfully in previous studies to monitor changes in perceptions of pain following exercise (Vaile, Gill, & Blazeovich, 2007).

### **Daily Analysis of the Lifestyle Demands of Athletes (DALDA)**

The questionnaire comprises 2 sections; part A identifies potential sources of stress (including home life, work, sleep and sports training), whilst part B allows individuals to rate stress reactions symptoms as worse than normal, normal, or better than normal. Several studies have successfully utilised the DALDA questionnaire to monitor fatigue and recovery (Hogarth, Burkett, & McKean, 2015; Robson-Ansley, Gleeson, & Ansley, 2009). Only results for part 'B' are reported here, after Halson et al., (2002) and Coutts et al., (2007) stated that there were only significant changes in the second part of the questionnaire following a period of intensified training.

### **Blood Sampling**

Approximately 8ml of blood was collected with stasis from the antecubital vein into plasma separation vacutainers (containing EDTA) for the purpose of assessing muscle damage and inflammation. Whole blood samples were assessed using a blood counter (AcT diff 5 CP, Beckman Coulter, High Wycombe, UK) to correct values for changes in plasma volume. The manufacturer reports the coefficients of variation for this system as <2 % for haematocrit and <1 % for haemoglobin within the reportable range. Blood was then centrifuged at 3000rpm for 8 minutes before being aliquoted and stored at -80°C for later analysis. Blood markers included creatine kinase-M (CK-M), C reactive protein (CRP), interleukin-6 (IL-6) and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and were all determined in duplicate.

### **CK-M**

Plasma CK-M concentrations were measured by simple step enzyme-linked immunosorbent assay (ELISA, Abcam, Cambridge, UK). The reported assay ranges are 54.3 – 268.9 U/L, the minimum detection concentration (MDC) is 0.014 U/L, and the human serum intra- and inter-assay CV are 3% and 9%, respectively.

### **CRP**

Plasma CRP concentration was determined using a quantitative sandwich (QS) enzyme linked immunoassay (ELISA) technique (Quantikine, R&D Systems Europe Ltd., Abingdon, UK). The limit of quantification (LOQ),

defined as the lowest concentration that could be distinguished from 0 was 7.8 pg/ml with an intra and inter-assay CV of 6.6 and 8.3% respectively.

#### **IL-6**

Plasma IL-6 concentration was determined by a QS-ELISA (Quantikine, R&D Systems Europe Ltd., Abingdon, UK). The limit of quantification (LOQ), defined as the lowest concentration that could be distinguished from 0 was 0.38 pg/ml. The serum intra- and inter-assay precision, determined by CV were 3.8 and 8.3% respectively.

#### **TNF- $\alpha$**

Plasma TNF- $\alpha$  concentration was measured by QS-ELISA (Quantikine, R&D Systems Europe Ltd., Abingdon, UK). The limit of quantification (LOQ), defined as the lowest concentration that could be distinguished from 0 was 0.52 pg/ml. The serum intra- and inter-assay precision, determined by CV were 4.9 and 9.9% respectively.

#### **Correction for haemoconcentration**

Bouts of acute exercise, such as long distance running, have been shown to produce a transient fluid shift out of the intravascular space, resulting in haemoconcentration (Kargotich, Goodman, Keast, & Morton, 1998). To minimise any confounding effects of the dynamic nature of plasma volume (Alis et al., 2015), changes in plasma volume were assessed using the following equation;  $\Delta PV (\%) = 100 \times ((HB_{pre}/HB_{post}) \times (100 - HTC_{post}) / (100 - HTC_{pre}) - 1)$  where PV is plasma volume, HB is haemoglobin and HTC is haematocrit. All blood markers were then adjusted using the following equation;  $(Biomarker)_c = (Biomarker)_u \times (1 + \Delta PV (\%) / 100)$  where c is corrected and u is uncorrected.

#### **Interventions**

##### **Whole Body Cryotherapy**

The WBC group was exposed to 2 cold treatments in a cryotherapy chamber (CryoClinics, London, UK). Participants (up to 2 at a time) spent 3 minutes in the chamber set to  $-85^{\circ}\text{C} \pm 5^{\circ}\text{C}$  followed by a 15 minute warming period in an ambient room before entering the chamber for a further 4 minute bout at  $-85^{\circ}\text{C} \pm 5^{\circ}\text{C}$ . During exposure participants were asked to walk around slowly while flexing and extending their elbows and fingers, and wore a pair of shorts, gloves, dry socks and shoes, a hat covering the ears and a mask to protect the nose and mouth. Before entering the chamber participants were asked to remove glasses, contact lenses and any jewellery or piercings.

##### **Cold Water Immersion**

Immediately after cessation of exercise participants sat in a mobile ice bath (iSprint Twin, iCool, Cranlea, UK) ensuring their lower limbs and iliac crest were fully immersed. Participants remained in the ice bath filled with

water cooled to 8 degrees ( $\pm 0.5^\circ$ ) for 10 minutes. The ice bath was connected to a chiller unit (MiCool, iCool, Cranlea, UK) so that water temperature could be monitored and maintained within the desired parameters for the duration of the treatment. During exposure participants wore shorts and immediately after they were asked to towel themselves dry and change into clean, dry clothing. This protocol is comparable to those utilised in other single exposure studies examining the effects of CWI on various measures of recovery (Bailey et al., 2007; Jakeman, Macrae, & Eston, 2009).

### **Placebo**

As it was not possible to blind participants to their recovery intervention, a placebo, rather than a control group was used. The phytochemicals found in Tart Montmorency cherry juice have previously been shown to reduce inflammation and improve muscle recovery following a marathon (Bell, McHugh, Stevenson, & Howatson, 2014). Therefore, the placebo group was informed that they were taking a tart cherry juice supplement for 5 days before the run, the day of the run and for 2 days after (8 days in total). Participants consumed 2 x 30 ml per day of a fruit flavored drink which did not contain any antioxidants or phytonutrients. Participants were asked to rest quietly for 10 minutes following completion of the run. It was hoped that the use of a placebo (sham) group would minimise associated placebo effects (i.e. effects of the treatment that were not related to the treatment itself) (McClung & Collins, 2007).

### **Statistical Analysis**

Confidence intervals (CI) and magnitude based inferences were calculated for each dependent variable using methods described by Batterham and Hopkins (2006). The smallest practically worthwhile effect for muscle function variables was the smallest standardised (Cohen) change in the mean: 0.2 times the between-subject SD for baseline values of all participants (Batterham & Hopkins, 2006). The smallest worthwhile change for muscle soreness and DALDA was a change in raw values of 1.0 and for blood parameters a factor of 1.1 was used (Hopkins, 2015). In order to account for large inter-individual differences in blood parameters, baseline values were used as a covariate. Qualitative descriptors relate to the likelihood of positive, trivial or negative outcomes. Clinical inferences were based on threshold chances of harm and benefit of 0.5 and 25% respectively. In cases where the inference was unclear, a beneficial inference was reported where the odds ratio of benefit/harm was greater than 66. In order to overcome heteroscedastic error, the analysis of dependent variables was conducted on log-transformed data (Nevill & Lane, 2007), except in the cases of muscle soreness and DALDA. Interval scaling makes it inappropriate to log-transform data for these variables (Nevill & Lane, 2007) so analysis was conducted on raw values. Each dependent variable was analysed using a published spreadsheet by Hopkins

(2015). Changes are reported as percentages for function variables, raw changes for perceptual variables and factor changes for blood markers.

## **RESULTS**

The outcomes for changes over time as well as group comparisons for all parameters can be seen in tables 2 and 3. The marathon resulted in decreases in muscle function, increases in circulating CK, increases in perceptions of soreness and alterations in a number of blood borne markers of inflammation.

### **Muscle Function**

A summary of the statistical analyses for the effect of each intervention on markers of muscle function can be seen in table 2.

#### **Peak Torque knee extension**

At baseline, the peak torque knee extension values were  $178.24 \pm 28.41$ ,  $195.33 \pm 29.92$  and  $203.72 \pm 39.47$  Nm for placebo, CWI and WBC respectively. Peak torque decreased in all groups at both time points post marathon. WBC was harmful at all time points in comparison to both CWI and placebo (Fig 1).

**Fig.1** Comparison of changes in PT extension at  $60 \text{ deg}\cdot\text{s}^{-1}$  as a percentage of baseline scores. Values are presented as mean  $\pm$  SD

### **MVIC**

At baseline, MVIC values were  $197.85 \pm 51.15$ ,  $221.81 \pm 37.48$  and  $228.60 \pm 54.68$  N for placebo, CWI and WBC respectively. Changes in MVIC were unclear or trivial in the CWI and placebo groups, whilst there were harmful changes in the WBC group. WBC was harmful compared to both CWI and placebo at all time points.

#### **Reactive Strength Index**

At baseline, RSI values were  $0.88 \pm 0.21$ ,  $0.89 \pm 0.30$  and  $1.03 \pm 0.29 \text{ m}\cdot\text{s}^{-1}$  for placebo, CWI and WBC respectively. RSI decreased in all groups from baseline to 24h, with unclear or trivial changes from baseline to 48h. WBC was harmful compared to CWI and placebo at all time points.

### **Perceptual Responses**

A summary of the statistical analyses for the effect of each intervention on perceptual responses can be seen in table 2.

#### **Perceived Muscle Soreness**

At baseline, soreness values were  $1 \pm 2$ ,  $1 \pm 1$  and  $1 \pm 1$  (VAS 0-10) for placebo, CWI and WBC respectively (Fig 2.). Perceptions of soreness increased in all groups from baseline to 24h, and in the placebo group from baseline to 48h. WBC was possibly beneficial compared to placebo at 48h, whilst all other group comparisons were trivial or unclear.

**Fig.2** Changes in perceptions of muscle soreness. Values are presented as mean  $\pm$  SD

## DALDA

At baseline, DALDA values were  $4 \pm 3$ ,  $1 \pm 2$  and  $4 \pm 4$  scores marked as worse than normal for placebo, CWI and WBC respectively. The number of scores marked ‘worse than normal’ increased in the CWI and placebo group at 24 and 48h, whereas the change was unclear at 24h and beneficial at 48h for WBC. At all time points, WBC was beneficial compared to CWI and unclear compared to placebo. CWI was harmful compared to the placebo at all time points.

Table 2. Change over time and group comparison outcomes for muscle function and perceptual responses.

		Changes over Time Mean <sup>a</sup> ; $\pm$ CL <i>Qualitative outcome</i>			Effects Mean <sup>a</sup> ; $\pm$ CL <sup>b</sup> <i>Qualitative Outcome</i>		
		Placebo	CWI	WBC	PL/CWI	PL/WBC	CWI/WBC
Peak Torque (%)	B – 24h	-3.7; $\pm$ 4.3 <i>Harmful*</i>	-4.1; $\pm$ 5.5 <i>Harmful*</i>	-10.7; $\pm$ 4.0 <i>Harmful***</i>	-0.4; $\pm$ 6.9 <i>Trivial*</i>	-7.3; $\pm$ 5.5 <i>Harmful**</i>	-6.9; $\pm$ 6.4 <i>Harmful**</i>
	B – 48h	-1.6; $\pm$ 4.0 <i>Trivial*</i>	-1.7; $\pm$ 6.5 <i>Harmful*</i>	-5.3; $\pm$ 4.7 <i>Harmful*</i>	-0.2; $\pm$ 7.4 <i>Trivial*</i>	-3.8; $\pm$ 5.8 <i>Harmful*</i>	-3.7; $\pm$ 7.5 <i>Harmful*</i>
MVIC (%)	B – 24h	1.7; $\pm$ 5.5 <i>Trivial**</i>	-0.7; $\pm$ 5.2 <i>Trivial*</i>	-10.1; $\pm$ 3.3 <i>Harmful***</i>	-2.4; $\pm$ 7.0 <i>Harmful*</i>	-11.7; $\pm$ 5.5 <i>Harmful***</i>	-9.5; $\pm$ 5.5 <i>Harmful**</i>
	B – 48h	3.4; $\pm$ 8.5 <i>Unclear</i>	1.1; $\pm$ 5.5 <i>Trivial*</i>	-8.0; $\pm$ 6.5 <i>Harmful**</i>	-2.2; $\pm$ 9.3 <i>Harmful*</i>	-11.0; $\pm$ 9.2 <i>Harmful**</i>	-9.0; $\pm$ 7.8 <i>Harmful**</i>
RSI (%)	B – 24h	-4.9; $\pm$ 9.3 <i>Harmful*</i>	-4.8; $\pm$ 13.0 <i>Harmful*</i>	-13.7; $\pm$ 10.1 <i>Harmful*</i>	0.1; $\pm$ 16.1 <i>Trivial*</i>	-9.2; $\pm$ 13.2 <i>Harmful*</i>	-9.3; $\pm$ 15.6 <i>Harmful*</i>
	B – 48h	2.9; $\pm$ 12.5 <i>Unclear</i>	2.6; $\pm$ 12.3 <i>Trivial*</i>	-5.8; $\pm$ 9.9 <i>Trivial**</i>	-0.3; $\pm$ 16.2 <i>Harmful*</i>	-8.4; $\pm$ 14.0 <i>Harmful*</i>	-8.2; $\pm$ 14.0 <i>Harmful*</i>
Muscle Soreness	B – 24h	2; $\pm$ 1 <i>Harmful**</i>	2; $\pm$ 1 <i>Harmful**</i>	1; $\pm$ 1 <i>Harmful*</i>	0; $\pm$ 2 <i>Trivial*</i>	-1; $\pm$ 2 <i>Unclear</i>	-1; $\pm$ 1 <i>Unclear</i>
	B – 48h	1; $\pm$ 1 <i>Harmful*</i>	0; $\pm$ 0 <i>Trivial***</i>	0; $\pm$ 1 <i>Trivial***</i>	0; $\pm$ 1 <i>Trivial**</i>	-1; $\pm$ 1 <i>Beneficial*</i>	-1; $\pm$ 1 <i>Trivial**</i>
DALDA	B – 24h	2; $\pm$ 3 <i>Harmful*</i>	4; $\pm$ 3 <i>Harmful***</i>	0; $\pm$ 2 <i>Unclear</i>	2; $\pm$ 4 <i>Harmful*</i>	-2; $\pm$ 3 <i>Unclear</i>	-4; $\pm$ 3 <i>Beneficial**</i>
	B – 48h	0; $\pm$ 3 <i>Harmful*</i>	1; $\pm$ 3 <i>Harmful*</i>	-2; $\pm$ 2 <i>Beneficial*</i>	1; $\pm$ 3 <i>Harmful*</i>	-2; $\pm$ 3 <i>Unclear</i>	-3; $\pm$ 3 <i>Beneficial**</i>

Qualitative outcome represents the likelihood that the true value will have the observed magnitude represented by the number of asterisks (\*) with \*possibly, \*\*likely, \*\*\*very likely and \*\*\*\* most likely.

<sup>a</sup>Mean represents the second named group minus the first named group.

<sup>b</sup>90%CL – add and subtract this number to the mean to obtain the 90% confidence limits for the true difference

## Blood Markers

A summary of the statistical analyses for the effect of each intervention on blood markers can be seen in table 3.

### **CK**

At baseline, CK values were  $31.2 \pm 18.0$ ,  $25.0 \pm 14.5$  and  $44.2 \pm 70.4$  U.L<sup>-1</sup> for placebo, CWI and WBC respectively. CK increased in all groups at both time points post marathon. WBC was harmful compared to CWI, and both cryotherapy interventions were harmful compared to placebo at 24h. All group comparisons from baseline to 48h were unclear.

### **CRP**

At baseline, CRP values were  $1625 \pm 3838$ ,  $586 \pm 378$  and  $553 \pm 573$  ng.mL<sup>-1</sup> for placebo, CWI and WBC respectively. CRP increased in all groups from baseline to 24h and in the CWI and placebo groups from baseline to 48h. WBC was harmful compared to CWI and placebo at 24h, but beneficial in the same comparisons at 48h. CWI was beneficial compared to placebo at both time points.

### **IL-6**

At baseline, IL-6 values were  $42.60 \pm 104.35$ ,  $75.93 \pm 157.27$  and  $17.81 \pm 33.51$  pg/ml for placebo, CWI and WBC respectively. IL-6 increased in all groups immediately post marathon, and remained elevated in the WBC group only at 24h post. From baseline to post, WBC was beneficial compared to CWI, CWI was harmful compared to placebo, and the comparison between WBC and placebo was unclear. At 24h WBC was harmful compared to CWI and placebo, whilst the comparison between CWI and placebo was unclear.

### **TNF- $\alpha$**

At baseline, TNF- $\alpha$  values were  $52.5 \pm 118.0$ ,  $327.6 \pm 928.1$  and  $58.9 \pm 119.4$  pg/ml for placebo, CWI and WBC respectively. TNF- $\alpha$  increased in all groups following the marathon. Comparisons between WBC and CWI were unclear at all time points, WBC was harmful compared to placebo immediately post, and CWI was harmful compared to placebo at all time points.

Where there are large differences in baseline values between groups (CRP, IL-6 and TNF- $\alpha$ ), this is attributed to one or two individuals who had values substantially greater than the normal range. However, as results are analysed as the difference between groups in change over time, these participants were not removed from the analysis.

Table 3. Change over time and group comparison outcomes for blood markers.

		<b>Changes over Time</b>			<b>Effects</b>		
		Mean <sup>a</sup> ; x/÷ CL			Mean <sup>a</sup> ; x/÷ CL <sup>b</sup>		
		<i>Qualitative outcome</i>			<i>Qualitative Outcome</i>		
		Placebo	CWI	WBC	PL/CWI	PL/WBC	CWI/WBC
CK	B – 24h	1.6; x/÷ 1.2 <i>Harmful****</i>	2.5; x/÷ 2.2 <i>Harmful***</i>	3.0; x/÷ 1.7 <i>Harmful****</i>	1.4; x/÷ 2.2 <i>Harmful*</i>	2.0; x/÷ 1.7 <i>Harmful***</i>	1.4; x/÷ 2.4 <i>Harmful*</i>
	B – 48h	1.5; x/÷ 1.2 <i>Harmful****</i>	1.7; x/÷ 1.5 <i>Harmful***</i>	1.5; x/÷ 1.5 <i>Harmful**</i>	1.1; x/÷ 1.5 <i>Unclear</i>	1.1; x/÷ 1.6 <i>Unclear</i>	1.0; x/÷ 1.7 <i>Unclear</i>
CRP	B – 24h	23.2; x/÷ 1.5 <i>Harmful****</i>	3.7; x/÷ 2.0 <i>Harmful***</i>	26.2; x/÷ 1.4 <i>Harmful****</i>	0.2; x/÷ 2.2 <i>Beneficial****</i>	1.5; x/÷ 1.6 <i>Harmful**</i>	8.1; x/÷ 2.2 <i>Harmful****</i>
	B – 48h	13.5; x/÷ 1.4 <i>Harmful****</i>	4.6; x/÷ 1.5 <i>Harmful****</i>	0.9; x/÷ 9.4 <i>Unclear</i>	0.4; x/÷ 1.7 <i>Beneficial***</i>	0.1; x/÷ 9.8 <i>Beneficial***</i>	0.2; x/÷ 10.0 <i>Beneficial**</i>
IL-6	B – Post	2.7; x/÷ 1.5 <i>Harmful****</i>	3.7; x/÷ 1.4 <i>Harmful****</i>	2.8; x/÷ 1.7 <i>Harmful***</i>	1.52; x/÷ 1.65 <i>Harmful**</i>	0.96; x/÷ 1.88 <i>Unclear</i>	0.63; x/÷ 1.78 <i>Beneficial**</i>
	B – 24h	0.9; x/÷ 1.3 <i>Unclear</i>	0.9; x/÷ 1.4 <i>Unclear</i>	1.2; x/÷ 1.2 <i>Harmful**</i>	0.92; x/÷ 1.50 <i>Unclear</i>	1.25; x/÷ 1.32 <i>Harmful**</i>	1.36; x/÷ 1.46 <i>Harmful**</i>
TNF- $\alpha$	B – Post	1.0; x/÷ 1.2 <i>Trivial*</i>	1.1; x/÷ 1.3 <i>Harmful*</i>	1.0; x/÷ 1.1 <i>Trivial**</i>	1.1; x/÷ 1.3 <i>Harmful*</i>	1.1; x/÷ 1.2 <i>Harmful*</i>	1.0; x/÷ 1.3 <i>Unclear</i>
	B – 24h	1.0; x/÷ 1.1 <i>Trivial**</i>	1.2; x/÷ 1.3 <i>Harmful*</i>	1.1; x/÷ 1.1 <i>Harmful*</i>	1.2; x/÷ 1.3 <i>Harmful*</i>	1.0; x/÷ 1.2 <i>Trivial*</i>	0.9; x/÷ 1.3 <i>Unclear</i>
	B – 48h	1.1; x/÷ 1.1 <i>Harmful*</i>	1.1; x/÷ 1.2 <i>Harmful*</i>	1.0; x/÷ 1.2 <i>Harmful*</i>	1.0; x/÷ 1.2 <i>Harmful*</i>	1.0; x/÷ 1.2 <i>Unclear</i>	0.9; x/÷ 1.3 <i>Unclear</i>

Qualitative outcome represents the likelihood that the true value will have the observed magnitude represented by the number of asterisks (\*) with \*possibly, \*\*likely, \*\*\*very likely and \*\*\*\* most likely.

<sup>a</sup>Mean represents the second named group minus the first named group.

<sup>b</sup>90%CL – times and divide the mean by this number to obtain the 90% confidence limits for the true difference

## DISCUSSION

The present study examined the efficacy of a single bout of whole body cryotherapy (WBC) or cold water immersion (CWI) on markers of recovery in trained endurance athletes following a marathon run. The marathon lead to modest alterations in muscle function, perceptions of muscle soreness and stress response symptoms, as well as increases in markers of muscle damage and inflammation. In terms of comparison between the different interventions, WBC was harmful for recovery of muscle function compared to CWI, with both cryotherapy groups being detrimental in comparison to the placebo. WBC was beneficial for limiting stress response symptoms and muscle soreness in comparison to CWI and the placebo, respectively. Both cryotherapy interventions lead to greater increases in CK than the placebo, with WBC harmful compared to CWI at 24h. There was little evidence of the cryotherapy interventions limiting inflammation based on IL-6 and TNF- $\alpha$ , and in some cases lead to greater increases. However, cryotherapy lead to the attenuation of increases in CRP.

WBC was detrimental to recovery of peak torque compared to both placebo and CWI at 24 and 48h; there were no differences between CWI and placebo. These results suggest that CWI may offer benefits compared to WBC for the recovery of peak torque, but that it is no more effective than a placebo intervention. In the case of MVIC

and RSI, WBC lead to greater decrements than both CWI and placebo, and CWI was also less effective than the placebo for recovery. The novel finding that cryotherapy is harmful for recovery in comparison to a placebo could be linked to an enhanced inflammatory response evidenced by an increase in pro-inflammatory markers (Hauswirth et al., 2011). Machado and colleagues (2016) have previously suggested that any CWI exposure below 10°C could be classed as 'severe' cold, with the potential to cause adverse effects that are interpreted by the body as noxious stimuli. Although muscle temperature was not recorded in the present study, it is possible that both an 8°C CWI exposure and a -85°C WBC exposure resulted in substantial reductions of muscle temperature that elicited a stress response in the body (Machado et al., 2016). This may explain the harmful effects of cryotherapy in this case.

The present study's results contrast with those from White, Rhind and Wells (2014) who reported that CWI (10min or 30min at 10°C) facilitated restoration of muscle performance in a stretch-shortening cycle following high-intensity sprint exercise. This study suggests that immersion at 8°C negatively impacts upon functional recovery after endurance exercise. The use of a placebo group in place of a control could explain the findings; research suggests that many of the hypothesised physiological benefits surrounding CWI are at least partly placebo related (Broatch, Petersen, & Bishop, 2014). Broatch and colleagues (2014) found that CWI was no more effective than a placebo immersion protocol at improving muscle recovery following high intensity exercise. They suggested that effective deception of participants is critical when using a placebo and that treatment belief is a powerful element (Beedie et al., 2017). Anecdotal evidence from the present study suggests that the placebo was administered effectively, and that participants believed in its efficacy.

Differences in muscle soreness between WBC and CWI were either trivial or unclear. These results are in contrast to Abaidia and colleagues (2016) who found that CWI resulted in lower soreness scores 48h post eccentric exercise in comparison to WBC. These differences may be explained by the warmer WBC protocol utilised in the present study (-85° versus -110° C), potentially indicating that warmer WBC temperatures are more effective at alleviating perceptions of soreness after exercise, compared to 'extreme cold' exposures (Machado et al., 2016). Alternatively, the use of a novel high intensity eccentric biased exercise protocol may have produced greater structural damage, secondary inflammation and stimulation of pain receptors than that seen in the present study (Clarkson & Hubal, 2002; Scholz & Woolf, 2002). The finding that CWI was not effective at reducing soreness compared to the placebo is in contrast to the majority of previous research on the topic. As already discussed, an immersion temperature of 8°C may have been suboptimal; exposure temperatures of between 10 and 15°C are more commonly cited in the literature (Machado et al., 2016) and



appear to be effective at reducing muscle soreness post exercise. Secondly, few studies investigating the influence of CWI on recovery have effectively blinded participants to their intervention group. The use of a placebo in the current study may negate some of the positive expectance effects attributable to the placebo effect (Broatch et al., 2014; McClung & Collins, 2007) and therefore offer a more robust examination of the effectiveness of CWI and WBC interventions used post exercise.

Cryotherapy had a negative effect on structural muscle damage assessed via circulating CK. Between baseline and 24h there was a harmful effect of WBC compared to CWI, and both cryotherapy treatments were harmful compared to the placebo. A recent study from Abaïdia and colleagues (2016) demonstrated a very likely large effect for CK in favour of CWI compared to WBC 24h post exercise, supporting the present study's indication that CWI may offer additional benefits over WBC for the attenuation of CK 24h post exercise. Cryotherapy lead to greater increases in leukocytes following the marathon (unreported data). It is plausible that leucocytosis lead to an increased breakdown of the sarcolemma that increased the efflux of CK in the cryotherapy groups in comparison to the placebo.

Cryotherapy is proposed to potentiate anti-inflammatory actions by decreasing peripheral blood flow (Mawhinney et al., 2017). The results from the present study do not support this hypothesis; despite WBC demonstrating a beneficial effect on IL-6 compared to CWI from baseline to post, when compared to the placebo, CWI and WBC were harmful and unclear, respectively. Similarly, WBC and CWI were harmful for changes in TNF- $\alpha$  with no clear differences between the cryotherapy groups. As previously stated, Machado et al., (2016) suggest that 'severe cold' immersion protocols (5-10°) can actually negatively impact upon recovery, by eliciting a cold related stress response. This in turn may increase markers of inflammation. However, CRP was the only marker where cryotherapy treatment had a positive influence compared to placebo. The seemingly equivocal results could be explained in part by different time courses of the markers; IL-6 tends to peak immediately post exercise (Bernecker et al., 2013; Clifford et al., 2016; Mündermann et al., 2016), whereas CRP normally continues to increase until 24h post exercise (Howatson et al., 2010). As such, cryotherapy applied post exercise may have been unable to attenuate increases in IL-6 but could positively impact upon the recovery of CRP.

Potential limitations of the current study should also be addressed. The cryotherapy chamber utilised during data collection was located a short distance off site, and as such, there may have been slight inconsistencies in the timing of the post exercise blood sample for participants in the WBC group compared to CWI and placebo. It is possible that the delay in sampling could have resulted in inflated pro-inflammatory values in the WBC group

for the post exercise samples. However, the factor-fold increases in IL-6 and TNF- $\alpha$  were still greater in the CWI group compared to WBC immediately post. Secondly, the WBC treatment temperature in the present study (-85°) was warmer than normally reported in the literature (-110 to -140° C); dictated by the minimum operating temperature of the cryotherapy chamber. Therefore, although the results reported here add to the current body of literature, the results cannot be generalised to colder exposure temperatures.

## **CONCLUSION**

In terms of comparison between cryotherapy modalities, with the exception of DALDA scores at 24 and 48h, CRP at 48h and IL-6 immediately following the marathon, WBC demonstrated an unclear, or negative impact on all markers at all time points compared to CWI. These findings contradict the widely held assumption that WBC can elicit enhanced recovery benefits when compared to more traditional cryotherapy applications such as CWI.

Secondly, with the exception of soreness at 48h for WBC and CRP (24 and 48h for CWI and 48h for WBC) the implementation of a cryotherapy intervention resulted in unclear, trivial or harmful effects for every outcome measure when compared to the placebo intervention. This lends further weight to the suggestion that therapeutic effects attributed to cryotherapy protocols could be a product of the placebo effect. Therefore, this highlights the need for future research to implement effective placebo interventions in place of control groups, or to at least take into consideration a measure of treatment belief when comparing different intervention strategies.

It is hoped that the findings from this study will help inform practice of athletes, practitioners and coaches in relation to post exercise recovery strategies. Further research is warranted to investigate the impact of CWI versus WBC on recovery following different exercise stresses, and to better understand the underlying physiological mechanisms.

## **Conflicts of Interest and Source of Funding**

No funding was received for this work. The authors declare no conflict of interest.

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